Analysis of 18s rDNA Sequence of Two Species of Family Convolvulaceae

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Abstract

Two species of the family Convolvulaceae namely *Ipomoea staphylina* and *Merremia aegyptica* were collected from different parts of Karnataka. The ribosomal DNA was isolated from the plant leaf sample. The 1.8 kb 18s rDNA fragment was amplified using high fidelity PCR polymerase. The PCR product was sequenced to obtain sequence of 18s rDNA region. The molecular data were submitted to NCBI GenBank and obtained accession numbers. *Ipomoea staphylina* – JX974574, *Merremia aegyptica* – JX 974575. For sequence comparison of these two plants with other closest Convolvulaceae members, NCBI GenBank BLAST data was taken. Sequence comparison by MEGA 5.05 reveals the evolutionary distance of each plant. The phylogenetic tree for these data was constructed.

KEYWORDS: Convolvulaceae, *Ipomoea staphylina*, *Merremia aegyptica*, 18s rDNA, NCBI GenBank.

INTRODUCTION

The Convolvulaceae includes approximately 1600-1700 species under 55-60 genera (Mabberley 1987). The family is nearly cosmopolitan in distribution but its members are primarily tropical plants, with many genera are endemic to tropical zones of individual continents (Austin 1998). A traditional placement for the Convolvulaceae in the order Solanales (Cronquist 1988, Dahlgren 1989 & Thorne 1992). Takhtajan (1997) placed this family under Convolvulales, due to the presence of articulate latex canals, intraxylary phloem position, seed and pollen morphology, not shared with other Solanales. The present study is to investigate phylogenetic interrelationship through 18s rDNA sequence analysis.

MATERIALS AND METHODS

Plant Material

Plants were collected from various regions of Karnataka. Table 1 showing the location and flowering season. The morphological and floral characters were studied using Flora (Gamble1986). The specimens collected for planting purpose were transferred into polythene bags and brought as quickly as possible to the selected field and planted.

DNA Extraction and 18s rDNA sequencing

DNA was extracted by CTAB method (Doyle, 1987) which was modified according to the samples. In brief, 100mg of each young leaf sample were transferred into mortar &

pestle. 750 µl of suspension buffer was added and crushed into fine paste. The extract was pipetted into a 2ml vial by using a tip that is cut at the bottom. 5µl of RNase were added and mixed thoroughly by inverting the vial. It was placed at 65^{o}C for 10 minutes with intermittent mixing. 1ml of lysis buffer was added, thoroughly mixed and kept at 65^{o}C for 15 minutes. The sample was cooled at room temperature and centrifuged at 13,000rpm at room temperature. The supernatant was collected and transferred into a 2ml vial and was loaded on to the spin column (600 µl each time) and centrifuged at 13,000 rpm for a minute at room temperature. The contents of the collection tube were discarded and the spin column was placed back in the same collection tube. 500 µl of wash buffer was added to the column and centrifuged at 13000 rpm for 1 minute at room temperature. The contents of the collection tube were discarded. The spin column was placed back in the same collection tube. The empty column was spin with the collection tube at 13000 rpm for 2 minutes at room temperature. The spin column was placed in a fresh vial. 50 µl of warm elusion buffer maintained prior at 65^{o}C was added into the spin column. The vial was kept along with the spin column at 65^{o}C for 1 minute and centrifuged at 13000 rpm for a minute at room temperature and DNA sample were collected. The DNA concentration was determined by both UV spectrophotometer and quantitative analysis on agarose gel. The 1.8 kb rDNA fragment was amplified using high–fidelity PCR polymerase. The PCR product was sequenced bi-directionally using the forward primer 5^{1}-GTAGTCATATGCTTGTCTC-3^{1} & reverse primer 5^{1}- GAAACCTTGTTACGACTT -3^{1}.

PCR Amplification and Purification

The reactions for PCR amplification were performed with a final volume of 50 µl, containing 26.5 µl ddH_2O, 10 µl 5X Taq Buffer (Mg2+), 6 µl dNTPMix (2.5 mM), 5 µl of the DNA template, 2 µl of each PCR primer (50 mol µl $^{-1}$), and 0.5 µl Taq DNA polymerase (5 U µl $^{-1}$). The amplification of 18S rDNA was performed with an initial denaturation at 95°C for 2 minutes, 35 cycles at 95°C for 1 minute, 55°C for 1 minute, 72°C for 4 minutes, and a final extension step at 72°C for 6 minutes. The PCR products were confirmed by electrophoresis on 1% agarose gel. The gels were stained with bromophenol blue and photographed by Bio-imaging system.

Identification software details

Phylogenetic Tree Builder uses sequences aligned with System Software aligner. A distance matrix is generated using the Jukes-Cantor corrected distance model. When generating the distance matrix, only alignment model positions are used, alignment inserts are ignored and the minimum comparable position is 200. The tree is created using Weighbor with alphabet size 4 and length size 1000.

Weighbor Tree

Weighbor is a weighted version of Neighbor Joining that gives significantly less weight to the longer distances in the distance matrix. The weights are based on variances and co-variances expected in a simple Jukes-Cantor model.

Jukes-Cantor Correction

The Jukes-Cantor distance correction is a model which considers that as two sequences diverge, the probability of a second substitution at any nucleotide site increases. For distance-based trees such as Weighbor, the difference in nucleotides is considered for the distance, therefore, second substitutions will not be counted and the distance will be underestimated. Jukes- Cantor created a formula that calculates the distance taking into account more than just the individual differences.

Bootstrap

Bootstrapping is a statistical method for estimating the sampling distribution by resampling with replacement from the original sample. In making phylogenetic trees, the approach is to create a pseudoalignment by taking random positions of the original alignment. Some columns of the alignment could be selected more than once or not selected at all. The pseudoalignment will be as long as the original alignment and will be used to create a distance matrix and a tree. The process is repeated 100 times and a majority consensus tree is displayed showing the number (or percentage) of times a particular group was on each side of a branch without concerning the subgrouping.

Sequence comparison and phylogenetic tree construction

The 18s rDNA sequences were aligned along with the other closest Convolvulaceae members after taking NCBI GenBank BLAST data. Phylogenetic tree constructed using MEGA 5.05(offline version).

18s rDNA sequencing : The 18s rDNA sequence fragments with the primers mentioned above were successfully amplified. The base size of *Ipomoea staphylina* was 700bp. The base size of *Merremia aegyptica* was 740bp. After sequencing the 18s rDNA and obtained accession numbers from National Center for Biotechnology Information-USA(Table2).

RESULT AND DISCUSSION

TABLE1: Showing place of collection & Flowering-Fruiting season.

Specimen	Location & collection date	Flowering & fruiting
Ipomoea staphylina Romer &Schultes	Gadikoppa Shimoga (January 2011)	Fl: December – March Fr: February- May
Merremia aegyptica (Linn.) Urban	Hosakerehalli Bangalore (November 2011)	Fl: October- December Fr: December – January

TABLE 2: Plant specimens and NCBI GenBank Accession numbers

Plant specimen	Voucher	GenBank Accession number
Ipomoea staphylina	BUBH 133/2011	JX974574
Merremia aegyptica	BUBH 189/2011	JX974575

Evolutionary Distance and Evolutionary relationships of Taxa : Comparing the 18S rDNA sequence obtained in this study. The results of the evolutionary distance shown in table 3. The results of the evolutionary distance and the ratio of sequence divergence shown in Figure 1. *Ipomoea staphylina* plant is seperated from the rest of the plant specimens. Further *Merremia aegyptica* is closely related to *Convolvulus arvensis* and *Ipomoea hederacea.* Next *Convolvulus arvensis* and *Ipomoea hederacea* plants are very close to each other. The evolutionary history was inferred using the Neighbor-Joining method (Saitou N. and Nei M.1987). The optimal tree with the sum of branch length = 0.03292904 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein J. 1985**)**. The tree is drawn to scale, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Jukes-Cantor method (Jukes T.H. and Cantor C.R.1969) and are in the units of the number of base substitutions per site. The analysis involved 4 nucleotide sequences. Codon positions included were 1st+2nd+3rd. All positions containing gaps and missing data were eliminated. There were a total of 693 positions in the final dataset. Evolutionary analyses were conducted in MEGA5(Tamura K. et al. 2011).

TABLE 3: Plant specimens, evolutionary distance and number of base pairs.

Plant specimen	Evolutionary distance	No of base pairs
Convolvulus arvensis	0.011737	1765
Ipomoea hederacea	0.013213	1750
Ipomoea staphylina	0.013213	700
Merremia aegyptica	0.011728	740

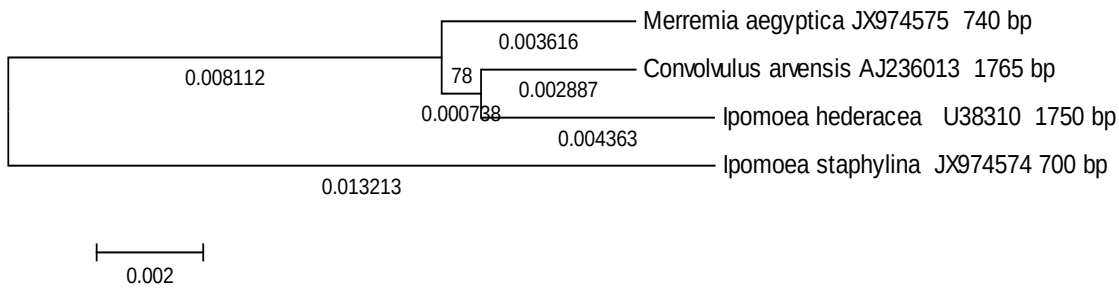

FIGURE 1: Phylogenetic tree constructed for Convolvulaceae members.

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